

## C L A I M S

- 5 1. A method for modifying a wild strain of an entero-  
invasive Shigella to produce a modified strain of  
Shigella that can be used for making a vaccine against  
the wild strain of Shigella characterized by the step of  
transforming the genome of the wild strain of Shigella so  
that it cannot substantially invade cells of a host and  
cannot spread substantially within infected cells and  
10 from infected to uninfected cells of the host and cannot  
produce toxins which will kill substantial numbers of the  
host's infected, as well as uninfected, cells.
- 15 2. The method of claim 1 in which the genome of the wild  
strain of Shigella is modified so that a first gene,  
coding for a protein necessary for the wild strain of  
Shigella to invade cells, as well as tissues, of the  
host, and a second gene, coding for a protein necessary  
for the wild strain of Shigella to spread within  
20 infected cells and between infected and uninfected cells  
of the host, are wholly or partly removed or permanently  
inactivated.
- 25 3. The method of claim 2 in which the Shigella is an S.  
flexneri and the first gene codes for the production or  
use of aerobactin by the S. flexneri.
4. The method of claim 3 in which the second gene codes  
for intra-intercellular spread.
- 30 5. The method of claim 2 in which the Shigella is an S.  
dysenteriae 1, the genome of which is modified so that a  
third gene, coding for the production or use of Shiga-  
toxin by the S. dysenteriae 1, is wholly or partly  
removed or permanently inactivated.
- 35 6. The method of claim 5 in which the first gene of the  
S. dysenteriae 1 codes for the production or use of  
enterochelin by the S. dysenteriae 1.

7. The method of claim 6 in which the second gene codes for intra-intercellular spread.
- 5 8. The method of claim 6 in which the first gene comprises the ent F, Fep E, Fep C and Fep D subunit genes of the enterochelin operon of the S. dysenteriae 1.
9. The method of anyone of claims 5-8 in which the first, second and third genes are mutagenized.
- 10 10. The method of anyone of claims 2-9 in which one or more of the genes are inactivated by allelic exchange with one or more in vitro mutagenized genes, especially mutagenized genes from which significant portions have been deleted and particularly mutagenized genes into which marker genes have been inserted.
- 15 11. A Shigella which has been modified by the method of anyone of claims 1-10 or is a descendant thereof.
12. A vaccine which has been made from the modified Shigella of claim 11.
- 20 13. A Shigella which is Shiga-toxin, particularly Shiga-toxin A.

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